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REMARKS

Interview Summaries

An in-person interview was scheduled and conducted on March 12, 2004. Participating in the interview were the Examiners Vera Afremova and Jon Weber; Applicant Dr. Kim Brouwer; Expert Dr. Ronald Borchardt (who is also a member of the Scientific Advisory Board and Board of Directors for Qualyst, Inc.); Mr. Scott Neuville, CEO, Qualyst, Inc., exclusive licensee to the technology and patent rights from assignee of record, The University of North Carolina at Chapel Hill; and applicants undersigned counsel, Arles A. Taylor, Jr. The claims and cited art of record were discussed. Applicants' arguments were found to be persuasive.

A telephone interview was conducted on March 24, 2004. Participating in the telephone interview were Examiner Vera Afremova and applicants undersigned counsel Arles A. Taylor, Jr. It was agreed that proposed amendments to claim 105 would be accepted and entered into the application, and that a draft Declaration under 37 C.F.R § 1.132 by applicant Xingrong Liu, Ph.D. would be accepted and entered into the prosecution of the subject application. Applicants submit formally herewith the discussed amendments of claim 105 and the discussed Declaration under 37 C.F.R § 1.132 by applicant Xingrong Liu, Ph.D.

Applicants wish to extend their most sincere thanks to Examiner Afremova and Examiner Weber for their time and consideration in participating in the interviews.

Status Summary

Claims 67-200 are now pending in the subject U.S. Continued Prosecution Application (CPA). Claims 67-200 as filed the instant CPA were subjected to a Restriction/Election Requirement. In a Response filed by facsimile on May 27, 2003, applicants elected the claims of Group III, claims 105-118, for prosecution at this time. Claims 105-118 have been examined.

Claims 105-118 have been rejected under U.S.C. Sections 102(a) and 103(a) based on the Liu et al. (1998) *Pharm. Sci.* 1:S-119 abstract (referred to hereinafter as Liu et al. [CC]), or on a dissertation by co-inventor Xingrong Liu entitled, "Sandwich-

Cultured Rat Hepatocytes: A Novel In Vitro Model To Study Hepatobiliary Disposition Of Substrates", filed with the U.S. Patent and Trademark Office (herein after the "Patent Office") in a supplemental filing dated May 28, 2003 and marked therein as "Exhibit B" (hereinafter the "Dissertation"), alone and in combination with other references.

Claims 105-118 have been rejected under 35 U.S.C. §102(b) or under 35 U.S.C. §103(a) based on Liu et al. (1997) *Pharm. Res.* 24:S-459 abstract (hereinafter referred to Liu et al. [EE]).

Claim 105 has been amended. Support for the amendment of claim 105 can be found in the subject U.S. patent application as filed at page 34, lines 7-9, wherein it is stated that: "Likewise, *in vitro* biliary clearance was calculated as the ratio of the amount excreted in the canalicular networks in the hepatocyte monolayers and the AUC in the incubation medium." The term "likewise" refers to the *in vivo* biliary clearance calculation, which was performed for comparison and was calculated as follows: "The *in vivo* biliary clearance was calculated in the Laboratory Examples as the ratio of the amount excreted into bile at time T and the plasma AUC between time 0 and time T." See page 33, line 21 through page 34, line 6. See also page 31, lines 4-7, wherein AUC is described as follows, in discussing the *in vivo* biliary clearance calculation: "AUC_{0-T} represents the area under the plasma concentration-time curve from 0 to time T (in minutes)".

Further, it is respectfully submitted that in view of the above-noted disclosure and discussion in the subject U.S. Patent Application as filed, the term AUC would be understood by one of ordinary skill in the art to refer to the following equation:

$$AUC = \int_0^T C\,dt, \text{ where C is concentration in medium.}$$

This equation is clearly set forth in the well-known textbook *Pharmacokinetics, Second Edition (Marcel Dekker, Inc. 1982)*, by Gibaldi and Perrier at pages 13 and 14. True and accurate copies of excerpts from this textbook are attached as **Exhibit 1**. The excerpts include pages 13 and 14.

Accordingly, applicants respectfully submit that the amendment is fully supported by the disclosure of the application as filed. No new matter has been added.

Applicants have carefully considered the outstanding rejections and the Patent Office's bases for these rejections and respectfully traverse the same as follows.

Response to Rejections of Claims 105-118 under 35 U.S.C. §§ 102(a) and 103(a) Based on the Dissertation or on Liu et al.[CC]

Claims 105-118 have been rejected under U.S.C. Sections 102(a) and 103(a) based on Liu et al. [CC], or on the Dissertation, alone and in combination with other references.

With regard to the publication of the Dissertation, applicants respectfully submit that the Dissertation was not publicly available as of the date of co-inventor's Liu's oral defense, as set forth on the Dissertation itself. However, assuming arguendo that the Dissertation was published prior to the March 17, 1999 priority date of the present U.S. patent application, and in further response to the rejection under 35 U.S.C. Section 102(a) based on Liu et al. [CC] or on the Dissertation, applicants submit the attached DECLARATION OF XINGRONG LIU PURSUANT TO 37 C.F.R. §1.132 (herein after the "Liu Declaration") regarding the Dissertation. Summarily, the attached Liu Declaration explains that while applicants LeCluyse and Brouwer are co-inventors for the subject matter disclosed and claimed in the present U.S. patent application, applicants LeCluyse and Brouwer are not co-authors of the Dissertation.

35 U.S.C. Section 102(a) states the following:

> A person shall be entitled to a patent unless --
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent,

35 U.S.C. Section 102(a) (1998) (emphasis added).

In view of the Declaration attached hereto, since the subject matter of the Dissertation is a portion of the presently claimed subject matter of the applicants, the Dissertation cannot therefore be cited as prior art under the provisions of 35 U.S.C. Section 102(a).

Particularly, applicant Liu could not have possibly described the subject matter of the invention in a printed publication (i.e. the Dissertation) before it was invented by applicants (i.e. LeCluyse, Brouwer, Liu), in accordance with the language set out above in 35 U.S.C. Section 102(a). This is because the facts are that the same Liu, who is the sole author of the Dissertation, is a co-inventor. It is impossible for an inventor to describe the invention in a printed publication before he/she has invented the invention. To show that these facts in the present situation are correct and hence 35 U.S.C. Section 102(a) is inapplicable, the Liu Declaration is attached.

Particularly, the attached Declaration establishes that co-inventors Brouwer and LeCluyse, provided input, support and guidance to the underlying research set forth in the Dissertation, as well as editorial and organizational input to the Dissertation itself. However, the writing of the Dissertation was a requirement upon co-inventor Liu to receive his doctorate from the University of North Carolina at Chapel Hill. Thus, co-inventors Brouwer and LeCluyse are not listed as co-authors of the Dissertation. The Declaration also confirms that co-inventors, Liu, Brouwer, and LeCluyse, are the sole co-inventors of the subject matter disclosed in the Dissertation and which is also part of the subject matter disclosed in the subject U.S. patent application.

Consequently, due the above comments and attached Declaration, it is respectfully submitted that the rejections of claims 105-118 under 35 U.S.C. Section 102(a) as being anticipated by and under 35 U.S.C. Section 103(a) as being unpatentable over the Dissertation has now been mooted. It is respectfully requested that the Dissertation as a reference be withdrawn, and hence, that the rejections be withdrawn.

Applicants now refer to the previously filed Declaration of Dr. Kim L.R. Brouwer Pursuant to 37 C.F.R. Sections 1.131 and 1.132. This Declaration is believed to establish that the intended subject matter of claims 105-118 was invented prior to the publication date of Liu et al. [CC]. The Declaration included the Dissertation as **Exhibit B**. The existence of the Dissertation predates the Liu et al. [CC] abstract and describes determination of a biliary clearance value for a xenobiotic compound in a hepatocyte culture as recited in claims 105-118.

Consequently, due the above comments and attached Liu Declaration, it is respectfully submitted that the rejections of claims 105-118 under 35 U.S.C. Section 102(a) as being anticipated by and under 35 U.S.C. Section 103(a) as being unpatentable over Liu et al. [CC] has now been mooted. It is respectfully requested that Liu et al. [CC] as a reference be withdrawn, and hence, that the rejections be withdrawn.

Allowance of claims 105-118 over the Dissertation and over Liu et al. [CC] alone or in combination with other cited references is therefore respectfully requested.

Response to Rejection of Claims 105-118 under 35 U.S.C. § 102(b) or 35 U.S.C. § 103(a) Based on Liu et al. [EE]

Claims 105-118 have been rejected under 35 U.S.C. §102(b) or under 35 U.S.C. §103(a) based on Liu et al. [EE]. Applicants respectfully submit that elected claims 105-118 are patentably distinguished over Liu et al. [EE] based on the following arguments.

The Liu et al. [EE] abstract describes the method of calculating the biliary excretion index based on the difference in uptake of substrate in standard buffer (HBSS) compared to calcium-free buffer at 10 minutes in Day 5 sandwich-cultured hepatocytes. Applicants respectfully submit that the use of the *in vitro* biliary clearance value is patentably distinguishable from the use of the biliary excretion index to characterize the way compounds are handled by the liver.

A biliary clearance value, as described in the present U.S. patent application, is calculated from fundamentally different data than those required for calculating the biliary excretion index. In order to collect these data, a different experimental design is required. The biliary excretion index is calculated from the difference in uptake of substrate in standard buffer (HBSS) compared to calcium-free buffer at 10 minutes in Day 5 sandwich-cultured hepatocytes. This difference (i.e., the mass of substrate that appears in the bile) comprises the numerator in both the biliary excretion index and the biliary clearance value calculation. However, the denominator differs for these two parameters. For the biliary excretion index, the denominator is simply substrate uptake in standard buffer. In contrast, for determination of a biliary clearance value, the area

under the curve (AUC), wherein the AUC represents the integral of xenobiotic concentration in the medium from time 0 to time T, serves as the denominator in the biliary clearance calculation. There is no disclosure in the Liu et al. [EE] abstract of any experimental design in which the calculation of AUC could be accomplished. Without this requisite information, biliary clearance cannot be calculated.

The biliary excretion index only reflects the fraction of the substrate accumulated in the hepatocyte that ultimately is excreted into bile. In essence, this parameter indicates the disposition of a compound only after it has been taken up into the cell. In contrast, the biliary clearance value determines the rate at which a compound will move from outside the cell into bile, without respect to which step (net uptake by the cell or movement from the cell interior into bile) might be the rate-limiting process. The distinction between the utility of the biliary excretion index and the biliary clearance value is important. A compound may have a high biliary excretion index even if biliary excretion is not an important route of elimination from the body. For some compounds, net uptake by the hepatocyte may be low (due to low uptake or significant efflux from the cell into the media), so that the liver does not contribute significantly to overall removal of the compound from the body. However, excretion of the compound from the cell into bile may be efficient. In this case, the biliary excretion index would be high, even though biliary excretion, from a biologic standpoint, would be unimportant. In contrast, the biliary clearance value would accurately characterize the behavior of the compound: a low degree of uptake into the hepatocyte will yield a low measure of biliary clearance regardless of how efficiently the compound is removed from the cell into bile. The presently claimed subject matter pertains to a method for determining the degree to which biliary clearance contributes to the removal of a xenobiotic from the systemic circulation. For example, this screening method can identify compounds that move rapidly from outside the hepatocyte into bile. In the context of identifying such compounds, the biliary excretion index can be misleading.

Physiological processes, such as clearance values, are additive and typically scale across species. This is not necessarily true for fractional excretion values. Compounds usually are categorized as low, intermediate or high clearance. Total

body clearance is the sum of hepatic clearance and clearance by all non-hepatic routes; in turn, hepatic clearance is the sum of metabolic clearance and biliary clearance. Total body clearance is a determinant of compound concentrations in blood that are produced by a given administration regimen for that compound. Hepatic clearance is a further determinant of systemic concentrations for compounds that are administered orally (as are most therapeutic agents) in that hepatic clearance can mediate loss of the compound before it appears in the systemic circulation (so-called "first-pass extraction" by the liver). It is critically important to identify compounds (potential drugs) that have a high hepatic clearance, as these will suffer a high degree of first-pass loss (and therefore may be undesirable as therapeutic agents). Compounds that have a high biliary clearance will have a high hepatic clearance and therefore, a large first-pass extraction; compounds with a high biliary excretion index may or may not have a high hepatic clearance.

The utility of the *in vitro* biliary clearance value, but inability of biliary excretion index, to predict *in vivo* biliary clearance is evident in the data submitted in the subject U.S. Patent Application Serial No. 09/527,352. See for example, Figures 6A and 6B. In Figure 6A, the biliary excretion index of methotrexate (open circle) is relatively high. However, the low *in vivo* biliary clearance value of methotrexate, as shown in Figure 6B of the subject U.S. Patent Application Serial No. 09/527,352, indicates that as methotrexate moves through the liver on any single pass, it is not rapidly excreted into bile. This can be the case if methotrexate is cleared predominantly by another route of elimination *in vivo* (e.g., non-hepatic routes). Biliary clearance also can be low if methotrexate is not taken up efficiently by the hepatocyte. Thus, the susceptibility of methotrexate to biliary excretion is low, but this would not be predicted by the biliary excretion index. In contrast, the low *in vitro* biliary clearance of methotrexate determined in the sandwich-cultured hepatocytes is predictive of the *in vivo* biliary clearance value.

Independent claim 105 recites in step (f) calculating a biliary clearance value. Turning now to the rejection of claim 105 based on Liu et al. EE, it is respectfully submitted that Liu et al. EE does not teach the calculating of a biliary clearance value. As such, Liu et al. EE cannot be seen to anticipate claim 105 under 35 U.S.C. § 102.

Liu et al. EE does not disclose each and every element of claim 105.

Turning now to the rejection of claim 105 under 35 U.S.C. § 103 based on Liu et al. EE, applicants respectfully submit that there is no motivation provided in Liu et al. EE to modify the teachings of Liu et al. EE to include a determination of AUC, wherein the AUC represents the integral of xenobiotic concentration in the medium from time 0 to time T. This is because there is no recognition in Liu et al. EE that one should account for sinusoidal uptake by incorporating such additional variables, since sinusoidal uptake can be a rate-limiting step in the overall biliary excretion process. Stated another way, there is no recognition by Liu et al. EE that sinusoidal uptake rather than canalicular excretion only, can be the rate-limiting step in the overall biliary excretion process. Thus, claim 105 is believed to be patentably distinguished over Liu et al. EE. Allowance of claim 105 is respectfully requested.

Claims 106-118 are dependent upon claim 105. Therefore applicants respectfully submit that dependent claims 106-118 are likewise patentably distinguished over Liu et al. EE. Allowance of claims 106-118 is also respectfully requested.

CONCLUSION

In light of the above Remarks, it is respectfully submitted that the present application is now in a proper condition for allowance and such action is earnestly solicited. If any minor issues should remain outstanding after the Patent Examiner has had an opportunity to study the above Remarks, the Patent Examiner is respectfully requested to telephone the undersigned patent attorney so that all such matters may be resolved and the application be placed in a condition for allowance without the necessity for issuance of another Official Action.

Deposit Account

The Commissioner is hereby authorized to charge any deficiencies of payment or credit any overpayments associated with the filing of this Amendment to Deposit Account No. **50-0426**.

Respectfully submitted,
JENKINS, WILSON & TAYLOR, P.A.

Date: March 26, 2004 By: Arles A. Taylor, Jr.

Arles A. Taylor, Jr.
Registration No. 39,395

Customer No. 25297

421/17/2 AAT/ptw

Enclosures

$$Cl_r = \frac{k_e X}{C} \qquad (1.24)$$

Recognizing that X/C is simply the apparent volume of distribution [Eq. (1.9)], we can shown that renal clearance is the product of the urinary excretion rate constant and the apparent volume of distribution:

$$Cl_r = k_e V \qquad (1.25)$$

All clearance terms can be expressed in terms of a rate constant and a volume.

An estimation of renal clearance by means of Eq. (1.23) may be misleading because like all rate processes in the body, renal excretion is subject to biologic variability. A more satisfactory approach is to plot urinary excretion rate versus drug concentration in plasma at the times corresponding to the midpoints of the urine collection periods (see Fig. 1.6). Since rearrangement of Eq. (1.23) yields

$$\frac{dX_u}{dt} = Cl_r C \qquad (1.26)$$

the slope of an excretion rate-plasma concentration plot is equal to renal clearance.

A second method for calculating renal clearance requires simultaneous collection of plasma and urine. Integrating Eq. (1.26) from t_1 to t_2 yields

$$(X_u)_{t_1}^{t_2} = Cl_r \int_{t_1}^{t_2} C\, dt, \qquad (1.27)$$

where $(X_u)_{t_1}^{t_2}$ is the amount of unmetabolized drug excreted in the urine during the time interval from t_1 to t_2 and $\int_{t_1}^{t_2} C\, dt$ is the area under the drug concentration in plasma versus time curve during the same time interval (see Fig. 1.7). Terms for area have units of concentration-time. A plot of $(X_u)_{t_1}^{t_2}$ versus $\int_{t_1}^{t_2} C\, dt$ yields a straight line with a slope equal to renal clearance.

Integration of Eq. (1.26) from time zero to time infinity, and rearrangement, gives an expression for the average renal clearance over the entire time course of drug in the body after a single dose:

$$Cl_r = \frac{X_u^\infty}{\int_0^\infty C\, dt} = \frac{X_u^\infty}{AUC} \qquad (1.28)$$

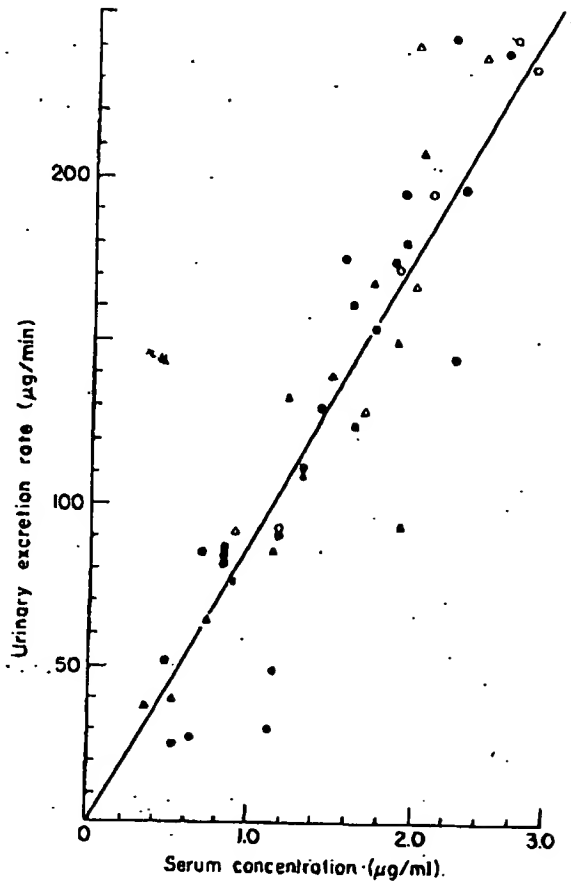

Fig. 1.6 Relationship between urinary excretion rates of tetracycline and serum concentrations of the drug determined at the midpoints of each urine collection interval after oral administration of a 250 mg dose to five healthy adults. Two different oral preparations (●, ▲) were given to each subject. The open symbols (○, △) denote the maximum excretion rate for each preparation. The data are described by Eq. (1.26); the slope of the line is equal to the average renal clearance of tetracycline in the group. (Data from Ref. 6.)

The term $\int_0^\infty C\, dt$ or AUC represents the total area under the drug concentration in plasma versus time curve plotted on rectilinear graph paper (see Fig. 1.7). This method has been used to estimate renal clearance (see Fig. 1.8) but is not ideal because it is difficult to collect urine for long periods to get an accurate estimate of X_u^∞, particularly for drugs with long half-lives.

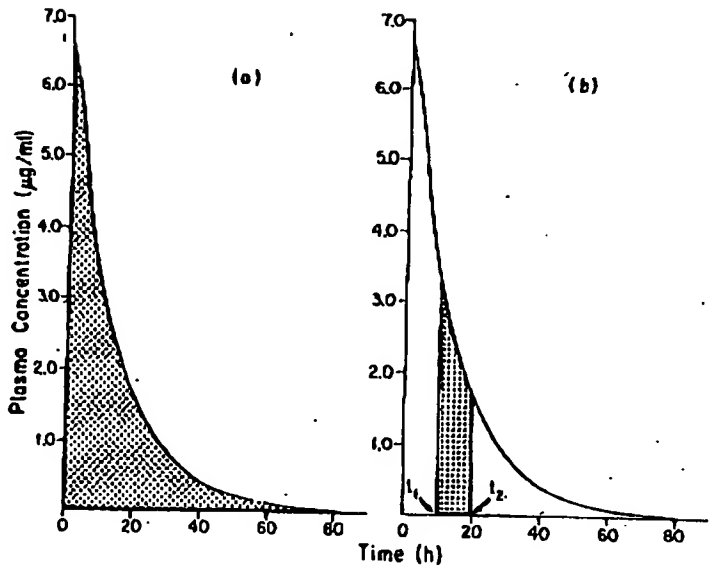

Fig. 1.7 Plots of drug concentration in plasma as a function of time after intravenous administration illustrating, by the shaded region, (a) $\int_0^\infty C\,dt$, the total area under the curve, AUC, and (b) $\int_{t_1}^{t_2} C\,dt$, the partial area under the curve from t_1 to t_2.

Use of Eqs. (1.27) and (1.28) for calculating renal clearance requires the measurement of areas under the drug concentration in plasma versus time curves. Several methods are available for determining the area under a curve. For each of these methods it is essential to obtain a sufficient number of blood samples to characterize adequately the curve or a portion thereof. A planimeter, which is an instrument for mechanically measuring the area of plane figures, is often used to measure the area under the curve (drawn on rectilinear graph paper). Another procedure, known as the cut and weigh method, is to cut out the area under the entire curve on rectilinear graph paper and to weigh it on an analytical balance. The weight thus obtained is converted to the proper units by dividing it by the weight of a unit area of the same paper. A third method to determine the area under the curve is to estimate it by means of the trapezoidal rule (see Appendix D). Other methods are described by Yeh and Kwan [7].

An exact mathematical method for determining the total area under the plasma concentration-time curve is to convert Eq. (1.10) to its exponential form and integrate over the time interval zero to infinity. Equation (1.10) expressed as natural logarithms is

$$\ln C = \ln C_0 - Kt \tag{1.29}$$

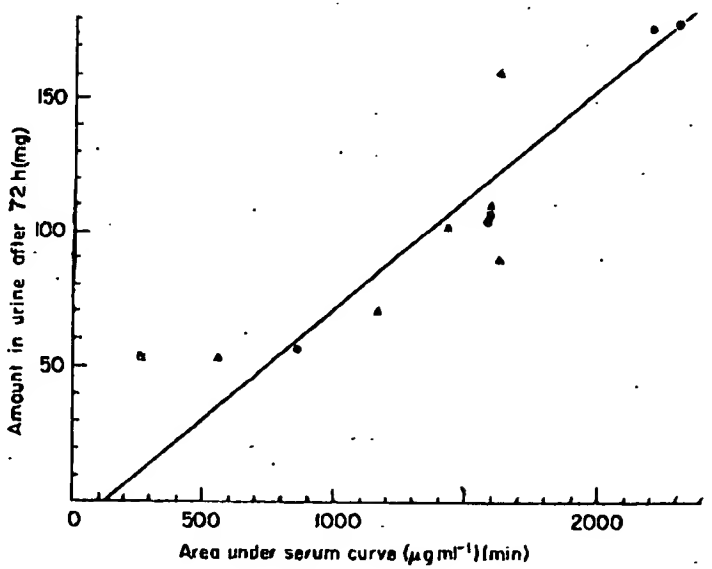

Fig. 1.8 Relationship between cumulative amount of tetracycline excreted after 72 h and the total area under the tetracycline concentration in serum versus time curve after oral administration of a 250 mg dose to five healthy adults. Two different oral preparations (●, ▲) were give to each subject. The data are described by Eq. (1.28); the slope of the line is equal to the average renal clearance of tetracycline in the group. (Data from Ref. 6.)

Therefore,

$$C = C_0 e^{-Kt} \tag{1.30}$$

Integration from time zero to time infinity yields

$$AUC = -\frac{C_0}{K} e^{-Kt}\Big|_0^\infty = \frac{C_0}{K} \tag{1.31}$$

Therefore, the total area under the plasma drug concentration-time curve is the plasma concentration at time zero, obtained by extrapolation, divided by the apparent first-order elimination rate constant of the drug. Since most drugs do not distribute instantaneously between plasma and tissues, Eq. (1.31) will usually underestimate the total area under the drug concentration in plasma versus time plot after intravenous administration. This error may be negligible or substantial, depending on the distribution and elimination characteristics of the drug.